EFFECT OF CALCIUM-ACTIVATED CHLORIDE CURRENT BLOCKADE ON THE DELAYED AFTERDEPOLARIZATIONS. SIMULATION STUDY.

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Abstract- **High intracellular calcium conditions cause a calcium-activated transient inward current (I_{ti}) that can provoke oscillations in membrane potential called delayed afterdepolarizations (DAD). The current I_{ti} comprises of the sodium-calcium exchange current (I_{NaCa}) and the calcium-activated chloride current ($I_{Cl\text{-}Ca}$). Lindblad, Murphey, Clark and Giles developed a mathematical model (LMCG model) of the rabbit atrial AP. In this study, a modified AP LMCG model that includes $I_{Cl\text{-}Ca}$ is used to evaluate the contribution of $I_{Cl\text{-}Ca}$ to develope DADs. Our results suggest that although I_{NaCa} is the main component of I_{ti} (65%), $I_{Cl\text{-}Ca}$ may play a significant role in DAD generation. Even more, the $I_{Cl\text{-}Ca}$ blockade could inhibit the DAD propagation and trigger activity associated to high $[Ca^{2+}]_i$ condition, in atrial tissue.**

Keywords **- $I_{Cl,Ca}$, chloride current, DAD, modelling**

I. INTRODUCTION

Delayed afterdepolarizations (DADs) are oscillations in membrane potential ocurring after completion of the action potential (AP). DADs are an important mechanism for cardiac arrhythmias [1, 2]. They usually occur under conditions in which $[Ca^{2+}]_i$ appears to be high. The mechanism underlying DAD is a transient inward current (I_{ti}) activated by spontaneous Ca^{2+} release from sarcoplasmic reticulum.

Although the ionic nature of I_{ti} is still subject to debate, recent experiments in ventricular and purkinje myocytes indicate that I_{ti} is composed by an electrogenic sodium-calcium exchange I_{NaCa} and a calcium-activated chloride current $I_{Cl\text{-}Ca}$ [1]. These studies suggest that the main component of I_{ti} is I_{NaCa} (about 60÷80 %) but the role of $I_{Cl\text{-}Ca}$ could be important.

$I_{Cl\text{-}Ca}$ is also known as the second component (I_{to2}) of a transient outward current (I_{to}) that is present in depolarization. $I_{Cl\text{-}Ca}$ magnitude is lower than I_{to1}, a potasium current, at normal conditions and slow rates. It is dependent on $[Ca^{2+}]_i$, time-independent and it presents a voltage-dependent outward rectification [4, 5, 6, 7]. I_{to2} is responsible for the maintenance of AP duration at high rates [5].

We developped a comprehensive model for the $I_{Cl\text{-}Ca}$, that was presented in a previous comunication [8].

This model has been introduced in the Lindblad, Murphey, Clark and Giles mathematical model (LMCG model) [3] of the rabbit atrial myocyte that reproduces the AP in a variety of conditions.

The present paper pretends to clarify the role of $I_{Cl\text{-}Ca}$ in DAD generation and in the induction of triggered activity.

II. METHODOLOGY

A set of mathematical equations describes the dependence of $I_{Cl,Ca}$ on intracellular Ca^{2+}, as well as on ionic concentrations. The current through Cl-Ca channels is described as a product of three terms: first term is expresed using the Goldmann-Hodgkin-Katz (GHK) equation, the second term (f_{Ca}) is the dependence on calcium and follows a Hill-type equation, and the last term (Rc) is the rectification exhibited by Cl-Ca channels, which is due to a voltage-dependent blockade caused by intracellular cations (Table 1) .

TABLE I
CALCIUM-DEPENDENT CHLORIDE CURRENT EQUATIONS

$$I_{Cl,Ca} = pcl \cdot f_{Cl,Ca} \cdot Rc \cdot \frac{v \cdot F^2}{R \cdot T} \cdot \frac{[Cl^-]_o \cdot e^{v \cdot F / R \cdot T} - [Cl^-]_i}{e^{v \cdot F / R \cdot T} - 1}$$

$$f_{Cl,Ca} = \frac{1}{1 + (K_{mCl,Ca} / [Ca^{2+}]_i)} \qquad Rc = \frac{1}{1 + e^{(v-44.4)/17.2}}$$

Model Parameters:
$\mathbf{K_{mCl,Ca}}$ = 150,2·10^{-3} mM/l **pcl** = 1,1712·10^{-3} nS

The mathematical description of $I_{Cl,Ca}$ was included into the atrial AP LMCG model. Software programs were written in ACSL language using Gear stiff algorithm to solve the nonlinear system of differential equations that results from the AP myocyte model. For studying propagation, the tissue is modeled as a one-dimensional 1-D segment (500 cells).

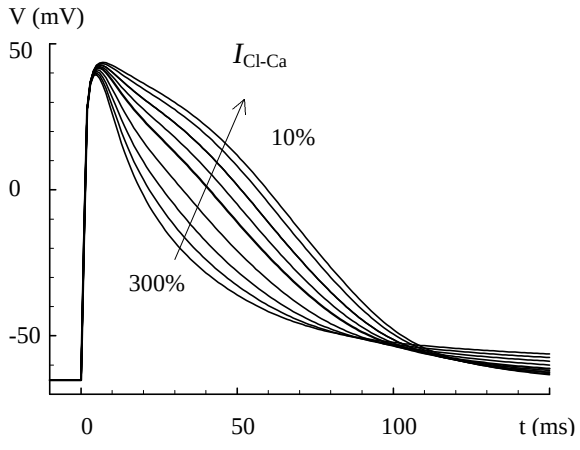

Figure 1. Effect of varying $I_{Cl\text{-}Ca}$ conductance (10, 25, 50, 75, 100, 150, 200, 250, 300 % of nominal) on model action potential morphology during stimulation at 2 Hz.

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Abstract

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The 1-D model was programmed in C++ language, the cable equation was solved using a central difference scheme in space and Crank-Nicholson method in time. Both, isolated myocyte and multicellular models, were implemented in a PC machine.

Protocol stimulation follows the nominal values of LMCG model for stimulation current (I_{st}) at 2 Hz rate [3].

III. RESULTS

Figure 1 shows the effect of varying the magnitude of the nominal $I_{Cl,Ca}$ conductance on action potential. Blockade of $I_{Cl,Ca}$ increase the action potential duration.

Figure 2 shows the time course of different membrane currents during the AP. Under normal conditions, $I_{Cl\text{-}Ca}$ is present mainly in depolarization.

Figure 3 shows the parameters f_{Ca} and Rc used to model $I_{Cl\text{-}Ca}$. In order to compare the model $I_{Cl\text{-}Ca}$ I-V plots with experimental data [5, 6], a train of voltage steps of 100 ms from – 70 to V was simulated at 0.1, 1 and 2 Hz. Plot C represents the normalized model results of peak current vs. experimental normalized data.

In order to study I_{ti} we considered DAD-generating conditions as intracellular calcium-overload. Calcium-overload is induced by an initial $[Ca^{2+}]_o$ higher than nominal.

In figures 4, 5 and 6, a train of 10 AP was stimulated but only the last AP was represented.

Figure 4 shows the membrane potencial at different initial $[Ca^{2+}]_o$ (A: 4, B: 5, C: 6 and D:7 mM/l) in control simulation (a) and when $I_{Cl\text{-}Ca}$ (b), I_{NaCa} (c) and both (d) are forced to zero at 4.8 s.

It can be observed that at higher calcium-overload conditions the delay between AP and DAD is lower ($t_4 < t_1$).

For $[Ca^{2+}]_o$ of 4 and 5 mM/l, the inhibition of $I_{Cl\text{-}Ca}$ has not significant effect on DAD amplitude (differences are below than 11 %). However, for $[Ca^{2+}]_o$ of 6 mM/l the inhibition of $I_{Cl\text{-}Ca}$ provokes a reduction of 58 % in DAD amplitude.

Figure 5 shows the case of an initial $[Ca^{2+}]_o$= 6 mM/l. In plot A can be observed the effect of $I_{Cl\text{-}Ca}$ blockade at 5.2 s

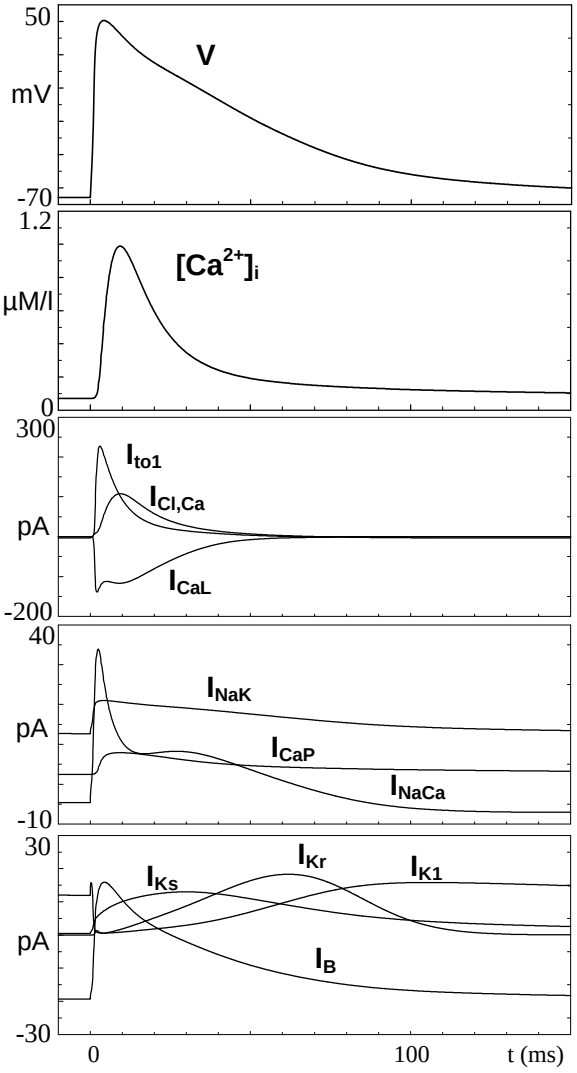

Figure 2. Currents and AP during stimulacion at 2Hz

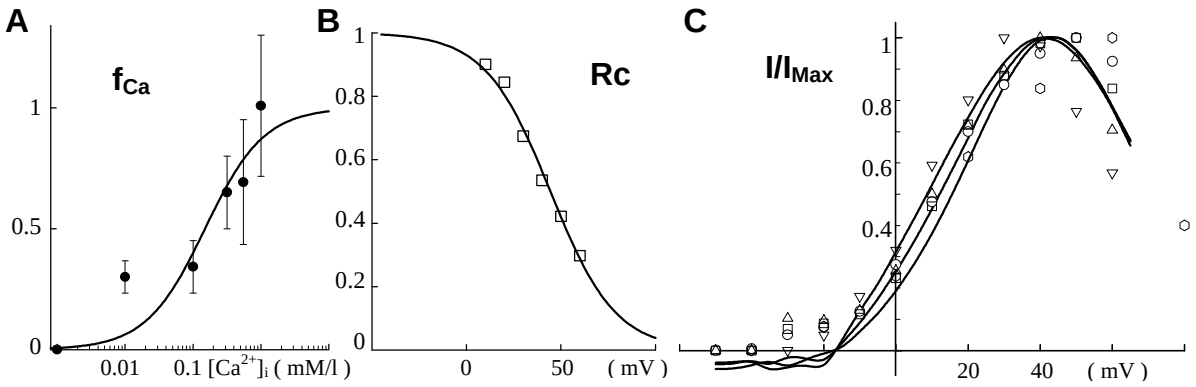

Figure 3. Parameters used to model calcium-activated chloride current ($I_{Cl\text{-}Ca}$). A: calcium-dependent open channels fraction. B: voltage-dependent blockade. C: I-V normalized relation. Data points represent experimental data.

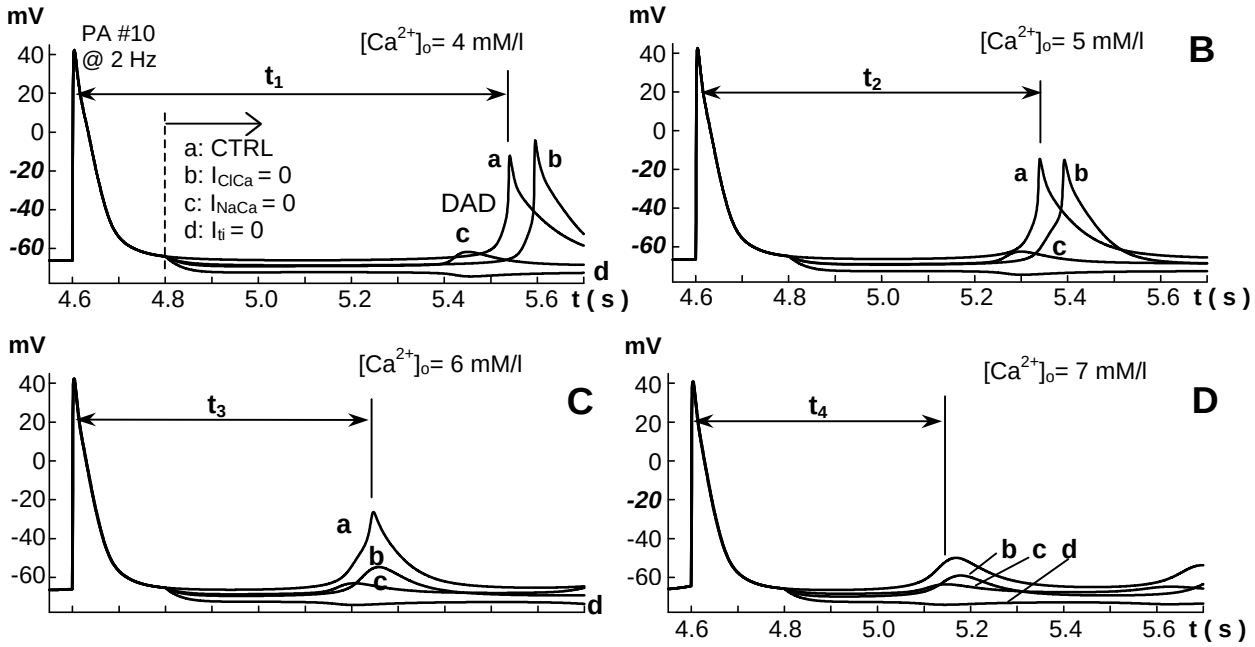

Figure 4. A secuence of 10 PA at 2 Hz was made. Calcium-overload is provoked by increasing $[Ca^{+2}]_o$ to 4, 5, 6 and 7 mM/l (nominal value is 2.5 mM/l). At 4.8 s we force to zero I_{ClCa}, I_{NaCa} and both (traces b, c, d) comparing them with control (trace a). The results in plot A and B show similar magnitude in traces a and b. The plot D shows a great attenuation of DAD traces. Only plot C seems to have a relevant difference between DAD amplitudes (traces a, b).

(b) vs. control (a) on DAD. In plot B can be observed that the contribution on I_{ti} of $I_{Cl\text{-}Ca}$ and I_{NaCa} is 35 % and 65 % respectivelly.

To study DAD propagation we use the 1-D model in a segment of 500 cells.

Figure 6 shows the membrane potential in the cell number 1, 125, 250, 375 and 500. As it can be observed, in plot A the DAD that appears in cell #1 is propagated like an AP in the cell #500. However, when I_{ClCa} is blocked, plot B, the oscillation in the membrane potential of cell #1 is not propagated like an AP in cell #500.

IV. Discussion

The main goal of the present study is to find out the role of I_{ClCa} as a component of I_{ti} on DAD formation and propagation as AP in the atrial tissue.

A mathematical model of the I_{ClCa} has been developed. Whenever possible, we use data directly measured on rabbit atrial cells to derive model parameters [5, 7]. When rabbit atrial data were not available to completely caracterize the current we use reliable data of dog and rabbit ventricular cells [4, 6].

The ionic nature of I_{ti} is still subject to debate. Several authors propose two ionic currents to contribute to I_{ti}: electrogenic I_{NaCa} and I_{ClCa} , but it is not clear in which percentage.

Recent data from Zygmunt el al. [1] indicate that I_{NaCa} component is the main responsible, representing about 60% of the total calcium-activated current at the resting potentials (I_{ti}). Other authors like Verker et al. [2] give values near to 80%.

Our results show that $I_{Cl\text{-}Ca}$ takes values near to 35% and $I_{Na\text{-}Ca}$ takes values near to 65% of the total calcium-activated current at the resting potentials (I_{ti}).

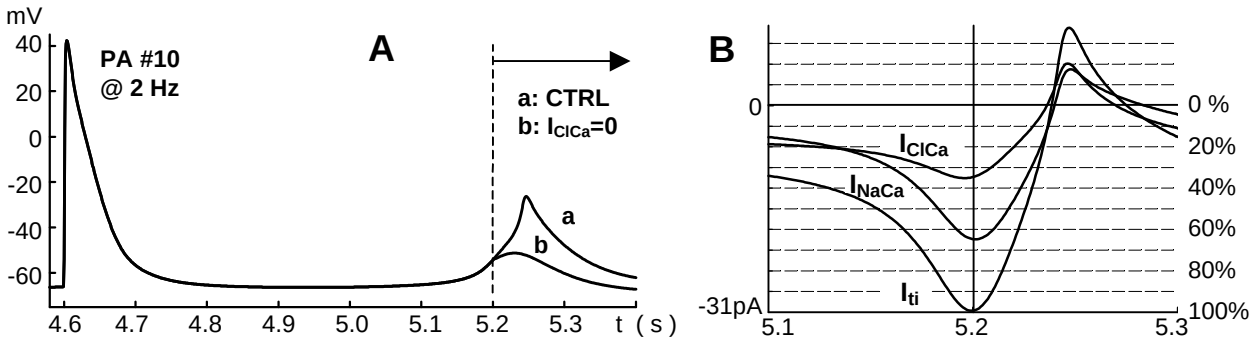

Figure 5. DAD after 10 PA @ 2 Hz, $[Ca^{2+}]_o$ = 6mM/l, CTRL and I_{ClCa}=0. A: DAD plot. B: I_{ti} CTRL composition %.

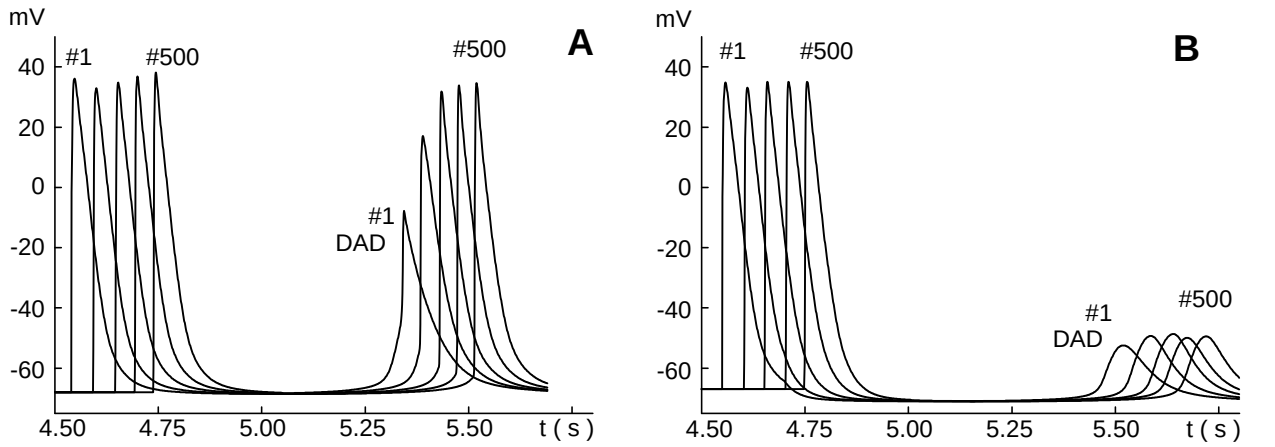

Figure 6. A secuence of 10 PA at 2 Hz in a 1-D segment of 500 cells was made. Calcium-overload is provoked increasing $[Ca^{+2}]_o$ to 7 mM/l. The plot **A** show the tissue propagation of the DAD. In the plot **B** we force to zero I_{ClCa} at t>4.6 s and AP < 10 % of maximum amplitude; in this case, DAD is lower than the subthreshold level for triggered AP.

V. Conclusion

Our results suggest that $I_{Cl\text{-}Ca}$ could play an important role in DAD generation mechanisms. The blockade of $I_{Cl\text{-}Ca}$ can reduce DAD amplitude to prevent DADs to reach the trigger threshold of action potentials.

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References

[1] Andrew C. Zygmunt, Robert J. Goodrow and Charlene M. Weigel. I_{NaCa} and $I_{Cl(Ca)}$ contribute to isoproterenol-induced delayed afterdepolarizations in midmyocardial cells. Am. J. Physiol. 1998; 275: H1979-H1992.

[2] Arie O. Verker, Marieke W. Veldkamp, Lennart N. Bouman and Antoni C.G.van Ginneken. Calcium-activated Cl^- current contributes to delayed afterdepolarizations in single purkinje and ventricular myocytes. Circulation 2000; 101: 2639-2644.

[3] Lindblad DS, Murphey CR, Clark JW, Giles WR. A model of the action potential and underlying membrane currents in a rabbit atrial cell. Am J Physiol. 1996;271:H1666-H1691.

[4] Mei Lin Collier, Paul C. Levesque, James L. Kenyon, Joseph R. Hume.Unitary Cl^- Channels Activated by Cytoplasmic Ca^{2+} in Canine Ventricular Myocytes. Circ. Res. 1996;78: 936-944.

[5] Zhiguo Wang, Bernard Fermini, Jianlin Feng and Stanley Nattel. Role of chloride currents in repolarizing rabbit atrial myocytes. Heart Circ. Phys. 1995; 37: 1992-2002.

[6] Andrew C. Zygmunt and W. R. Gibbons.Properties of the Calcium-activated Chloride Current in Heart. J. Gen. Phys. 1992; 99: 391-414.

[7] Dayue Duan, Stanley Nattel. Properties of Single Outwardly Rectifying Cl^- Channels in Heart. Circ. Res. 1994; 75: 789-795.

[8] Gomis-Tena J, Saiz J.Role of Ca-activated Cl currents in the heart: a computer model. Computers in Cardiology 1999; 26: 109-112.

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